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Short Communication: Optimazing culture and differentiation L6 cell, C_2C_{12} cell and primary myoblast cells culture

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[1]Division of Physiology, Departement of Basic Science, Faculty of Medicine, Universitas Padjadjaran. Jl. Sukarno-Hatta Km 21, Jatinangor, Sumedang 45363, West Java, Indonesia. ♥email: ronny@unpad.ac.id

[2]Central Laboratory, Universitas Padjadjaran. Jl. Sukarno-Hatta Km 21, Jatinangor, Sumedang 45363, West Java, Indonesia

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Abstract. *Lesmana R, Goenawan H, Tarawan VM, Setiawan I, Hidayat A, Supratman U. 2018. Short Communication: Optimizing culture and differentiation of L6 cell, C_2C_{12} cell, and primary myoblast cells culture. Cell Biol Dev 2: 51-54.* Alteration in skeletal muscle function and structure could result from many chronic diseases, drug treatment, or physical activity. Therefore, understanding the molecular mechanism of skeletal muscle diseases or the adaptation process will be very important and beneficial. In most cases, for example, during aging, the imbalance between anabolic and catabolic processes could initiate a decreased muscle mass and change its fiber structure. Unfortunately, there is no suitable animal model for study, and animal model development is time-consuming at some point. Next, it is good to study and elaborate a model on disease mechanisms, drug targets, and hormonal effects in skeletal muscle, which is not easy and tricky. Finally, the promising methods to study molecular biology properties of muscle are using *in vitro* systems utilizing 2 cell lines derived from mouse (C2C12 myoblast cells) and rat (L6 myoblast. Another establishment technique is using mixed and isolated single fiber primary myocytes culture.

Keywords: Cell line, C2C12, L6 myoblast cell, primary myocytes culture

INTRODUCTION

Alteration in skeletal muscle function and structure often occurs due to different chronic diseases, including cancer, heart failure and diabetes, aging, hormonal imbalance, and genetic myopathies (Lin et al., 2018; Demontis et al., 2013). The inflammatory cytokines are observed due to an imbalance between anabolic and catabolic processes (Demontis et al., 2013). There are many unknown mechanisms of hormonal regulation in controlling the physiological function of skeletal muscle, like thyroid hormone, mechanism of Emery Dreyfuss, Duchenne muscular dystrophy (DMD), exercise regulation, etc. (Bloise et al. 2018).

Limitations in utilizing animal models or performing experiments in vivo using mice, rats, and rabbits lead researchers to use cell lines for study. Experiments using *in vitro* models, like muscle culture systems, may become a very good solution (Liu et al., 2017; Rovetta et al., 2013). Unfortunately, there is limited knowledge to study using mature muscle cell culture derived from myoblast cell culture. and elaborate more on the mechanism of disease, drug target, and hormonal effects in skeletal muscle, and it is not easy and tricky (Rovetta et al. 2013; Koenig and Smith 1985). However, to better understand the disease mechanism in skeletal muscle, the right approach other than in *vivo* experiments using mice and rats is needed. Besides that, the animal ethical issues recommended reducing, refining, and resizing the number of animals used in the experiments as motivation to use the cell line as the best option to work with for study.

Setting an experiment using an in vitro system is one solution whenever there are difficulties in using mice or rats as animal models. Moreover, to set skeletal muscle in vitro experiments, better knowledge about muscle cell line characteristics is needed. Therefore, this study established the *in vitro* study using 2 cell lines derived from mouse: C2C12 myoblast cells and rat: L6 myoblast cells and mixed and isolated single fiber primary myocytes culture.

MATERIALS AND METHODS

Cell culture L6 cells

Rat L6 myoblast derived cell line was a more suitable model for thyroid hormone (TH) study (Koenig and Smith 1985). Therefore, L6 myoblast-derived cells were maintained at 37º C, with 5% CO_2 in DMEM supplemented with 10% Fetal Bovine Serum (FBS) and 100 µg/ml penicillin-streptomycin antibiotic. After 3 days of proliferation with DMEM contained with 10% FBS, the medium was changed with DMEM contained with 2% horse serum (HS) for 5-7 days to induce myotubes formation.

Cell culture C_2C_{12} cells

Mouse C_2C_{12} myoblasts were maintained under standard conditions (37 °C, humidified atmosphere, and 5% CO2) in Dulbecco's Modified Eagle's Medium (DMEM) high-glucose supplemented with 10% Fetal Bovine serum (FBS) and 100 µg/ml penicillin-streptomycin antibiotic. Like the Rat L6 differentiation protocol, cells were cultured and incubated for 5 days in differentiating medium (DM) with reduced serum content into 2% FBS. The myotubes were then fixed in absolute methanol for 10 min, stained with 10% Giemsa reactive, and observed using light microscopy.

Primary skeletal muscle culture cell

For four weeks, old Wistar rats were used in the experiment; muscles were excised and cleaned briefly from surrounding connective tissues. Then, muscle tissue was minced with sharp scissors and digested with protease XIV (p5147, Sigma Aldrich, USA). Several centrifugations separated the cells from muscle fiber fragments and tissue debris. After several layers fractioned count by Percoll density, cells were plated at 2.10^4 cells/cm^2 on a 2 cm Petri disc coated with poly-l-lysin and used alpha Modified Eagle Minimum essential. Primary culture myoblast cells were maintained at 37ºC with 5% CO_2 for 48 hours. Finally, to differentiate myoblast cells into myocytes, serum in the medium for differentiation was changed into 2% horse serum and maintained for 5 days (Ono et al. 2015).

RESULTS AND DISCUSSION

Proliferation and differentiation in L6 Cell

L6 cells were seeded with 4.10^4 cells/cm^2 density and followed up its progress using light microscope observation. Most of the cell shape was round at the proliferation state, and there was no long structure. However, after 5 days of differentiation, there are long tubes formation as a sign of myotubes formation of more than 65% in one field inspection. (Figure 1).

Proliferation and differentiation in C_2C_{12} Cell

Next, 5.10^4 cells/cm^2 of C_2C_{12} myoblast cells were seeded in 6 well plates. During proliferation, most of the cell shape was round and no long myocyte structure. However, five days after differentiation began, long structures as characteristic of myocyte cells were observed in >80% cell culture (Figure 2).

Proliferation and differentiation in primary myoblast culture cells

Primary myoblast cells were plated at 2.10^4 cells/cm^2 on the Petri disc. Within 3-4 days, the primary cell showed a growing process. Most of the cell shape is round and cylindrical at the proliferation state. After differentiation for 8-10 days, there was a shift that >90% of myoblast cells were transformed to myotube cell shape with characteristic has long tube structure form (Figure 3).

Discussion

Myopathic changes are observed in 30-80% of patients with hypothyroidism. Patients with more severe or longstanding untreated hypothyroidism are more likely to develop clinically significant muscle disease. Physiological aging also can alter muscle structure and functions (Lin et al., 2018). Four types of hypothyroid myopathy are found: Hoffmann syndrome, Kocher-Debre-Semelaigne syndrome, atrophic form, and myasthenic syndrome (Udayakumar et al. 2005; Vasconcellos et al. 2003). Those are an example of muscle diseases with an unclear mechanism; possibly, the physiological changes appear before or after the structural adaptation of skeletal muscle. Mechanisms of underlying pathological or physiological conditions in skeletal muscle can be studied easier using an *in vitro* system.

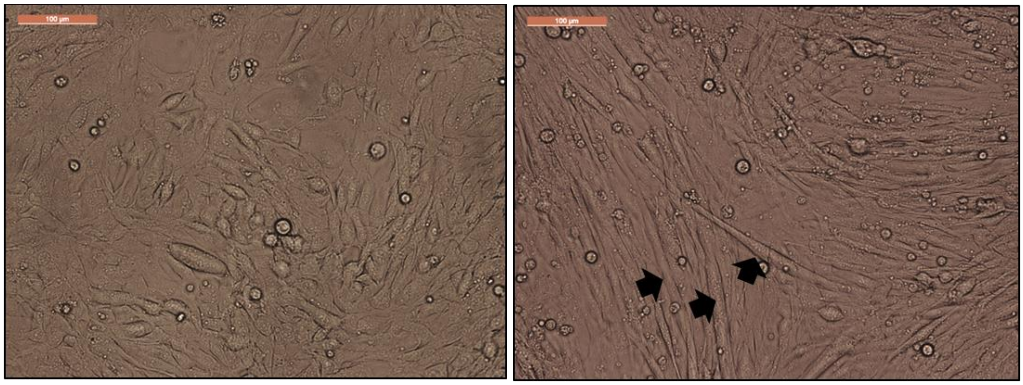

Figure 1. The different appearance between myoblast (Day 1; *left*) and after serum in the medium was adjusted and induced cell to myotubes (Day 5; *right*) in L6 cells

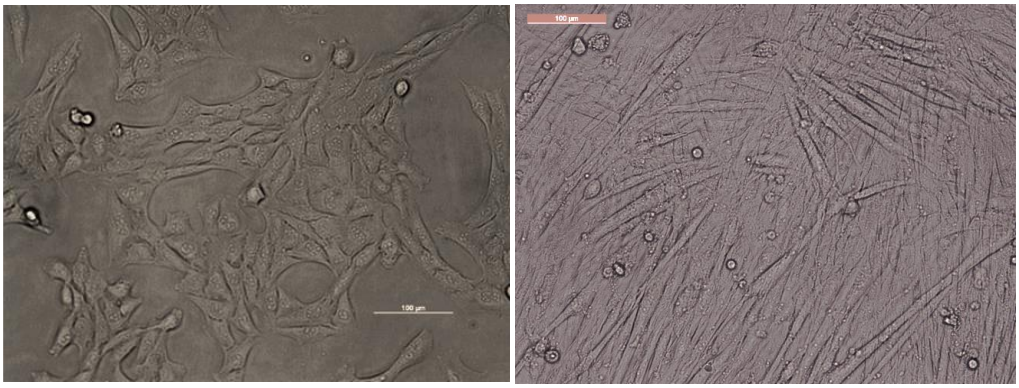

Figure 2. The different appearance between myoblast (Day 1; *left*) and after serum in the medium was adjusted and induced cell to myotubes (Day 5; *right*) in C2C12 cells

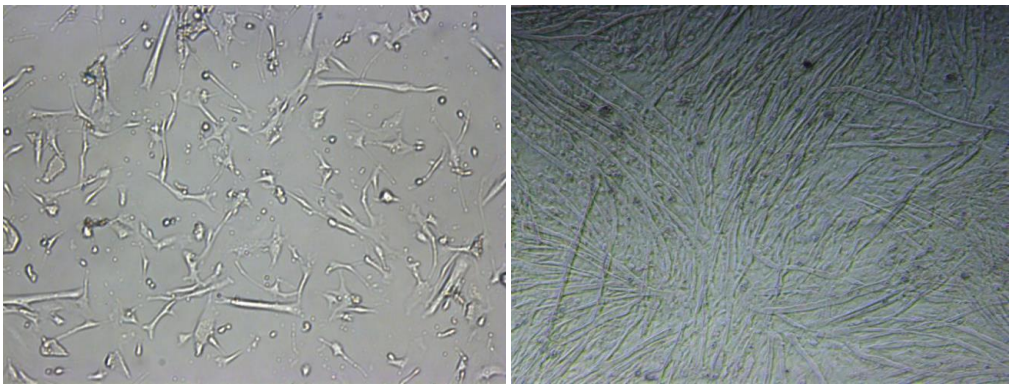

Figure 3. The different appearance between myoblast at day 2 (*left*) and myotubes at day 6 (*right*) in rat primary myoblast culture cell. The Source of muscle is the soleus muscle

Several types of immortalized muscle cell lines can be used for experimenting. The L6 cells are derived from rat myoblast, and the C_2C_{12} cells are derived from mouse myoblast; both cells are the most common cell line utilized for the experiment. Immortalized L6 cells and C_2C_{12} cells are cultured in myoblast form; under a specific condition, the cell will proliferate and differentiate (Rovetta et al., 2013; Koenig and Smith, 1985). An important key to getting long-lasting results is knowing every specific change during this process very well. At the end of the differentiation state, it is common to see a mixed population of myoblast and myotubes. Many researchers used arabinose to eradicate the myoblast from the plate culture to purify myotubes from myoblast. Next, it was possible to characterize these cells, mimic situations like in vivo experiments and set them according to the preferred experiment.

The limitation of the study utilizing L6 and C_2C_{12} cells are immortalized cells, which do not 100% reflect the physiological type of muscle function. However, primary myoblast culture or single fiber culture derived from any muscle can be used to overcome these limitations. Furthermore, primary myoblast culture showed a higher response to hormonal treatment, chemical or manipulation, and its signaling was closer to the *in vitro* experiment results (Iovine et al., 2012; Minami et al., 2011). Finally, optimizing protocol and conditions for in vitro experiments will help us to get the best results in our setting experiment. Therefore, L6 cell, C_2C_{12} cell, and primary myoblast cultures cell could be the answer for studying muscle physiology and pathology in mice and rats.

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